Platelet-rich plasma preparation using three different centrifugation methods: A comparative study

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Background
Platelet-rich plasma (PRP) is a platelet concentrate contained in a small volume of plasma, it has been a promising option in the last decade to treat different dermatology diseases, such as alopecia, skin ulcers, melisma, and burns due to the high concentration of growth factors that stimulate tissue repair and regeneration. The current study aims to compare the yield of platelets comparing different centrifugation methods, which are double-spin high-centrifugation force, double-spin low-centrifugation force, and single-spin system correlating each with the growth-factor concentration.

Patients and methods
This study was performed on 30 healthy individuals (12 males and 18 females), their age ranged from 17 to 49 years old. From each individual, 20 ml of venous blood was collected for both basal blood-cell count and for PRP preparation. The PRP samples were prepared by differential centrifugation methods through one of three different procedures using the laboratory centrifuge (Eppendorf 5810 R). Growth factors, including vascular endothelial growth factor, platelet-derived growth factor, and matrix metalloprotein-9, were measured in PRP samples using enzyme-linked immunosorbent assay technique.

Results
The current study showed that the double-spin low-centrifugation method increased the platelet count by 140% than the baseline value, with significant fold rise than in the single-spin and the double-spin high-centrifugation method, meanwhile, there is a statistically significant increase in the corresponding growth factors.

Conclusions
Double-spin low-centrifugation method and single-spin centrifugation provide better platelet yielding with efficient growth-factor concentrations.

Keywords:
centrifugation, growth factors, platelet-rich plasma

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Introduction

Platelets are cytoplasmic fragments of megakaryocytes, formed in the marrow, ~2 μm in diameter, its cytoplasm is abundant in alpha, dense, and lambda granules, which are rich in growth factors essential for acceleration of tissue-repair process [1]. Platelet-rich plasma (PRP) refers to a platelet concentrate in a small volume of plasma, with healing components such as growth factors and a diverse family of immunomodulatory proteins [2].

PRP is obtained from the blood-centrifugation process, where a concentration is three-to-five times than the basal platelet concentration, depending on the rotation force, duration of centrifugation, and the types and concentration of platelet activators and growth factors [1]. Variables such as the time elapsed between the activation and the application of PRP may also influence its quality and efficiency in therapy [3].

PRP was first used in therapeutic purposes in the 80s, by Matras[2] when she described the use of fibrin glue as a substance with sealing functions that helped repair of tissues in various oral and maxillofacial surgical procedures, followed by another trial, in 1986 by Marx when he used PRP for bone replacement after surgery [4]. Since these trials, the use of PRP is of growing interest in clinical procedures and research such as sports medicine, dental interventions, and plastic and orthopedic surgeries [5].

Centrifugation speed and duration is a crucial step to standardize the methodology; however, several centrifugation methods have been described [2,3,6–9].

Although the currently published literature described numerous methods of centrifugation, these methods can

be grouped into two large groups: single centrifugation and double centrifugation, the latter being the most used [10]. The most widely used double-centrifugation methods are by Sabarish *et al.* [4], also, Perazzi *et al.*[9] reported a single-spin method that aims to preserve the platelet integrity to achieve the maximum amount of growth factor after activation and degranulation of the platelets, and the double-spin high-centrifugation method that is the most widely used in our country (150 and 250 g) [8].

In the present prospective study, we aimed to compare three different methods of PRP preparation, which are the double-spin low centrifugation, the single-spin method, and the double-spin high-centrifugation method.

Patients and methods

This study was conducted on 30 healthy volunteers (12 males and 18 females), their age ranged from 17 to 49 years old, they were recruited according to the selection-inclusion criteria with no history of smoking, alcohol intake, or any systemic disorders that may affect the basal platelet count or the targeted growth-factor concentration from the Outpatient Clinic of the Dermatology and Andrology Department, Benha University. The demographic data and the clinical presentations of the studied population are described in the supplementary table. The study was performed after taking informed consent from the patients and volunteers and after permission from the Ethical Committee of Faculty of Medicine, Benha.

Platelet-rich plasma-preparation techniques

From each patient, 20 ml of venous blood was collected, divided into 2 ml, was added to a vacutainer containing EDTA disodium for complete cell count, and 18 ml added to 2 ml of acid citrate dextrose and were gently sloped several times to mix the blood with the anticoagulant solution and then divided into three samples (6 ml each) to prepare PRP by different centrifugation powers in a single-donor model.

The PRP samples were prepared by differential centrifugation methods through one of three different procedures: double-spin low centrifugation, single-spin centrifugation, and double-spin high-centrifugation methods using the laboratory centrifuge (Eppendorf 5810 R, Hanau, Germany) at a constant temperature of 22°C (Table 1).

After centrifugation, the platelet-containing plasma layer was separated without the buffy coat that is rich in white-blood cells, then transferred to a clean plain tube for further concentration, resulting in a pellet of PRP in the bottom and a supernatant of platelet-poor plasma, which is removed totally to measure the platelet count in the remaining pellet.

Growth-factor assay

This study was based on measuring vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF-α), as platelet-enrichment index, and matrix metalloprotein-9 (MMP-9) as a marker for white-blood-cell contamination. Samples of previously prepared PRP from the 30 volunteers were analyzed using enzyme-linked immunosorbent assay techniques (supported by Sigma Aldrich cat no. V7259 (Sigma Aldrich: Missouri, PO Box 14508, St. Louis, MO, 68178. USA), 127464-60-2, ABC1451, respectively) according to the manual instructions using (Stat Fax 2100, USA).

Study's outcome indices

The primary outcomes in this study were to assess the platelet recovery, the fold increase of the platelets, and the platelet concentration that were calculated as parameters for characterizing the performance of each centrifugation power and method based on the following equations.

Platelet recovery (%): volume of PRP obtained (ml)×platelet concentration in PRP (g/l).

Net volume of whole blood-collected (ml) ×platelet concentration in whole blood (g/l).

Concentration factor

Platelet concentration in PRP (g/l).

Platelet concentration in whole blood (g/l).

Statistical analysis

Data were analyzed using IBM SPSS advanced statistics version 20 (SPSS Inc., Chicago, Illinois, USA). Qualitative data were expressed using numbers and frequencies. Quantitative data were expressed using mean ± SD or median (range) as appropriate and number (percentage) for categorical variables. One-way analysis of variance and comparisons of statistical significance between patients' means followed by post-hoc test was expressed as *P* value (significance considered if $P < 0.0001$). Pearson correlation analysis was conducted to analyze the linear correlation between blood-cell concentrations and the χ^2 test was used to analyze the difference between groups regarding nonparametric variables.

Table 1 The used three centrifugation protocols for platelet-rich plasma preparation among the studied group (*n*=30)

Centrifugation protocols	Separation spin		Concentration spin	
	Rate (rpm)	Time (min)	Rate (rpm)	Time (min)
Method 1 (Marx *et al.*)	1000	4	800	9
Method 2 (Perazzi *et al.*)	100 G (945)	10	-	-
Method 3	150 G (1170)	10	250 G (3700)	15

Results

The association analysis showed no statistically significant difference between males and females (P = 0.152) in terms of platelet preparation using the different methods. The platelet counts in double-spin low-centrifugation force were 368.17 µl, while in single-spin centrifugation were 358.40 µl, and in double-spin, high-centrifugation method was 264.43 µl with a significant increase in platelet count in both the first and second methods compared with the third method (Table 2).

The results also elucidated that platelet count as the total concerning the age shows high significance between the different age groups to produce PRP (P = 0.03).

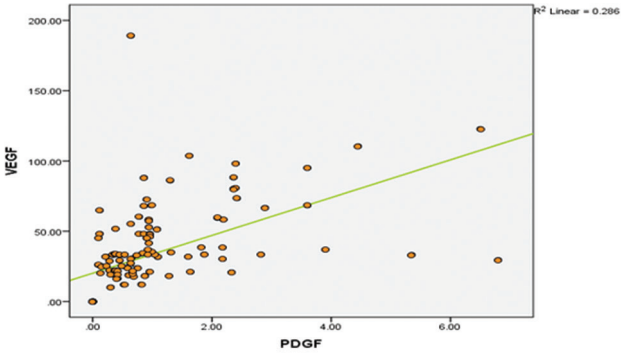
The results demonstrated that VEGF mean was 64.4 ± 26.3 ng/ml using the first centrifugation method, 32.3 ± 15.6 ng/ml using the second method, while was 35.2 ± 31.5 ng/ml in the third method, with a statistically significant difference between the first and the second methods (P = 0.0001), and between the first and the third methods (P = 0.0001), but there was no statistically significant difference between the second and the third methods (P = 0.958).

Using the first centrifugation method, PDGF concentration was 2.2 ± 1.7 ng/ml, but it was 0.81 ± 0.65 ng/ml using the second method, and was 0.75 ± 0.61 ng/ml using the third method. The results showed that there was a statistically significant difference between the first and the second method (P = 0.0001), and between the first and the third method (P = 0.0001), but there was no statistical significance between the second and the third method (P = 0.999).

Regarding the growth factor MMP-9, the mean concentration using the first method was 2.4 ± 3.1 ng/ml, but was 17.7 ± 7.2 ng/ml after using the second method, and was 10.9 ± 8.0 ng/ml with the third method. There was a statistically significant difference between the three used centrifugation methods (P = 0.0001).

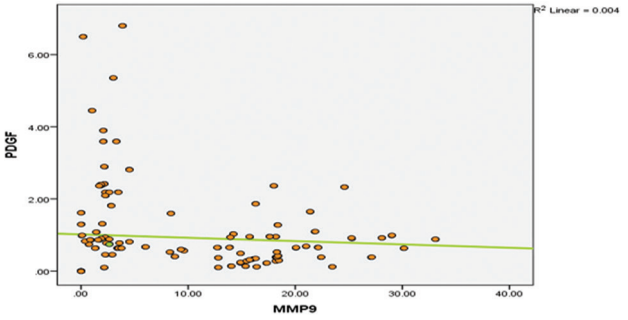
There was a statistically significant positive correlation between PDGF and VEGF (r = 0.286) (Fig. 1); controversially, there was a negative correlation between MMP-9 and PDGF (r = 0.004) (Fig. 2), and VEGF (r = 0.766) (Fig. 3).

Figure 1



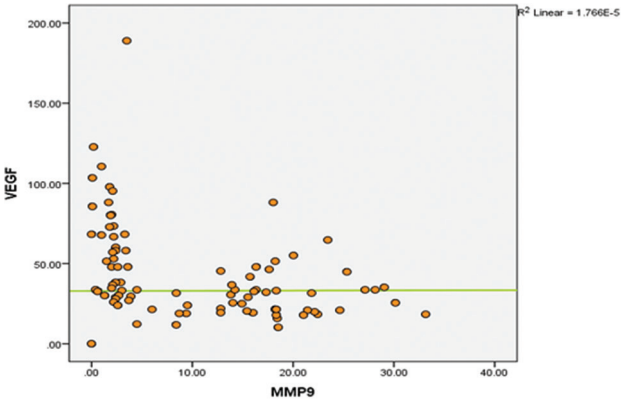
Correlation between VEGF and PDGF among the studied population (*n*=30). PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor.

Figure 2



Correlation between PDGF and MMP-9 among the studied population (*n*=30). MMP-9, matrix metalloprotein-9; PGDF, platelet-derived growth factor.

Figure 3



Correlation between VEGF and MMP-9 among the studied population (*n*=30). MMP-9, matrix metalloprotein-9; VEGF, vascular endothelial growth factor.

Table 2 Mean platelet count, platelet yield and platelet recovery in the three methods of centrifugation

	Mean platelet count (×10³/µl)	Mean platelet recovery (%)	Platelet yield	*P*
Whole blood	261.97	-		
Method 1	368.17	16.37±2.87	1.407	0.0001
Method 2	358.40	15.82±3.15	1.367	0.001
Method 3	264.43	12.11±4.44	1.033	1.00

There was a statistical significance between VEGF concentration and platelet count (P = 0.043), but no statistical significance between both PDGF and MMP-9 and platelet count (P = 0.087, 0.57, respectively).

Discussion

The use of PRP for therapeutic purposes has been dramatically increased in various clinical and therapeutic settings [1]. It has been proved that PRP has a potent effect on the healing process in both soft and hard tissues owing to the concentration of various growth factors in PRP concentrate [2]. Several methodologies have been described for PRP preparation based on different centrifugation protocols [3,6,11,12].

The present study aimed to elucidate the effect of three different centrifugation methods on the platelet count in PRP concentrate reflected by the concentration of various growth factors and both platelet recovery and platelet-concentration indices.

The current study was conducted on 30 volunteer individuals using three centrifugation protocols, including double-spin low centrifugation, single-spin centrifugation, and double-spin high-centrifugation methods.

The results of the current study showed no statistical significance regarding the sex with the mean of platelet concentration by the three methods (P = 0.738, 0.457, and 0.136, respectively), also, there was no statistically significant difference between the age and the mean of platelet concentration by the three methods (P = 0.397, 0.109, and 0.287, respectively). On the other hand, Weibrich *et al.*[13] in their study demonstrated that platelet concentrations in the whole blood and PRP were slightly higher for women than for men.

Clinical outcomes have shown that the age is not a major limitation when using platelet and plasma-based products for the treatment of different dermatological pathologies, and major age-specificand sex-specific differences in individual growth-factor concentrations were not found [14].

As regards the different centrifugation methods, the double-spin low-centrifugation method showed a 140% increase in platelet count than the baseline value. These results are in agreement with Sabarish *et al.* [4], who demonstrated a 160% increase in basal platelet using the initial protocol by Marx, although Fréchette *et al.*[14] reported a higher increase up to 200–300%, this difference may be explained by the ethnic variation and the initial volume for PRP preparation.

The difference in the result could be due to that the samples in the current studies were withdrawn on acid citrate dextrose anticoagulants, Araki *et al.*[11] used EDTA as an anticoagulant and their results reported 70–80% platelet recovery than the basal value.

Besides, the present study was based on avoiding taking the buffy-coat layer during PRP preparation, which leads to loss of part of the platelet in the buffy coat. Similar results were found with a 2.2-fold increase in platelet count. However, leukocyte-rich PRP was with platelet count seven-fold increase in the previous study performed by Melo *et al.* [3].

On the other hand, the results of the current study using a single-spin method showed a 1.5-fold rise in the baseline platelet count. These results were matched with Mazzucco *et al.*[7] and Perazzi *et al.* [9], who reported a twofold rise in the baseline platelet count.

As regards the double-spin centrifugation, the results of the current study showed about a one-fold rise in the baseline platelet count, which was different from previous studies by Perazzi *et al.*[9] and Nofal *et al.* [8], which reported a 4–4.5-fold rise in baseline platelet count. This can be explained that a portion of the platelets is lost with the dense-packed red blood cells during the first spin and the second spin, then clumping and/or disintegration of the platelet occur. When disintegration of the platelet occurs before PRP activation, the growth factors are released in an inactive state that may not influence the process of wound healing.

The results of the current study verified that the single-spin and the double-spin low-centrifugation methods were superior to the double-spin high-centrifugation method as regards the percent increase of platelet count than its baseline value, and preserving platelet integrity, meanwhile, the double-spin low-centrifugation method showed better results than the single- spin centrifugation method in the absence of platelet adsorption to the surface of the erythrocytes, as happens in the single-spin method. This was in agreement with other studies that reported that the double-spin low-centrifugation provides better platelet yield than the single-spin method [9,15].

The strength of the current study was in confirming the effect of variable centrifugation method on platelet yield in PRP concentrate based on measuring the concentration of the corresponding growth factors, such as VEGF and PDGF, which are involved in the healing process; meanwhile, by measuring MMP-9, which is a catabolic cytokine proved in other studies by Kobayashi *et al.*[16] and Yin *et al.*[17] to be positively correlated with neutrophils and used as an estimate of white blood cell count, especially neutrophil in the sample.

The concentration of the growth factors (PDGF and VEGF) in our study was statistically higher than the baseline concentration, in agreement with the study indicating the increase of growth factors in PRP when compared with baseline values by Arora *et al.* [18].

In the first method, VEGF has the highest mean (64 000 ng/ml), while PDGF (2.21 ng/ml) and MMP-9 (2.4 ng/ml) have the lowest concentration mean. That is because it preserves the integrity of the platelet until activation and degranulation of its growth factors to get the maximum amount could be achieved.

Meanwhile, VEGF showed a positive correlation with PDGF (r = 0.286), but a negative correlation between MMP-9 and PDGF (r = 0.004).

These results are in agreement with Weibrich *et al.*[13] in their study who illustrated that there is some degree of correlations regarding the growth-factor levels, however, there is substantial individual variation in the growth-factor content and using PDGF as a predictive estimate for some of the growth factors.

Conclusion

The results of the study concluded that the double-spin low-centrifugation method (1000 rpm for 4 min and then 800 rpm for 10 min), and the single-spin method (945 rpm) capture and concentrate platelets and growth factors more efficiently compared with the double-spin high-centrifugation method and may be able to provide an optimal method for the preparation of P-PRP for clinical application.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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